

WHAT IS CLAIMED IS:

1. Chimeric DNA encoding a membrane bound protein comprising in reading frame:

DNA encoding a signal sequence;

5 DNA encoding a non-MHC restricted extracellular binding domain of a surface membrane protein that binds specifically to at least one ligand, wherein said ligand is a protein;

DNA encoding a transmembrane domain; and

10 DNA encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system,

wherein said extracellular domain and cytoplasmic domain are not naturally joined together and said cytoplasmic domain is not naturally joined to an extracellular ligand-binding domain, and when said chimeric DNA is expressed as a membrane bound protein in a selected host cell under conditions suitable for expression, said chimeric DNA initiates signalling in said host cell.

2. DNA according to Claim 1, wherein said cytoplasmic domain is selected from the group consisting of the CD3 zeta chain, the CD3 eta chain, the CD3 gamma chain, the CD3 delta chain, the CD3 epsilon chain, the gamma chain of a Fc receptor and a tyrosine kinase.

3. DNA according to Claim 2, wherein the cytoplasmic domain is the gamma chain of the FcεR1 receptor.

30 4. DNA according to Claim 1 wherein said extracellular binding domain is the heavy chain of an immunoglobulin, by itself or in conjunction with a light chain, or truncated portions of said heavy chain

and/or said light chain containing ligand binding activity.

5. DNA according to Claim 1 wherein said extracellular domain is CD8.

5 6. DNA according to Claim 1 wherein said extracellular domain is CD4.

7. DNA according to Claim 1, wherein said extracellular domain is a single-chain antibody, or portion thereof.

10 8. DNA according to claim 7, wherein said single-chain antibody recognizes an antigen selected from the group consisting of viral antigens and tumor cell associated antigens.

15 9. DNA according to claim 8 wherein said single-chain antibody is specific for the HIV env glycoprotein.

10. DNA according to claim 9 where said cytoplasmic domain is zeta.

20 11. DNA according to Claim 1, wherein said transmembrane domain is naturally joined to said extracellular domain.

12. DNA according to Claim 1, wherein said transmembrane domain is naturally joined to said cytoplasmic domain.

25 13. An expression cassette comprising a transcriptional initiation region, DNA according to Claim 1 under the transcriptional control of said

transcriptional initiation region, and a transcriptional termination region.

14. An expression cassette according to Claim 11, wherein said transcriptional initiation region ~~is~~  
5 functional in a mammalian host.

15. A retroviral RNA or DNA construct comprising an expression cassette according to Claim 14.

16. A cell comprising DNA according to Claim 1.

17. A cell according to Claim 16, wherein said  
10 cytoplasmic domain is the CD3 zeta chain.

18. A cell according to Claim 17, wherein said extracellular domain is the heavy chain of an immunoglobulin, by itself or in conjunction with a light chain, or truncated portions of said heavy chain  
15 and/or said light chain containing ligand binding activity.

19. A cell according to Claim 17 wherein said extracellular domain is CD8.

20. A cell according to Claim 17, wherein said extracellular domain is CD4.

21. A cell according to Claim 16, wherein said transcriptional initiation region is functional in a mammalian cell and said cell is a mammalian cell.

22. A cell according to Claim 21, wherein said  
25 mammalian cell is a human cell.

23. A cell according to claim 16 wherein said cell is a hematopoietic stem cell.

24. A chimeric protein comprising in the N-terminal to C-terminal direction:

a non-MHC restricted extracellular binding domain of a surface membrane protein that binds specifically to at least one ligand;

a transmembrane domain; and

a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system,

wherein said extracellular domain and cytoplasmic domain are not naturally joined together, and said cytoplasmic domain is not naturally joined together to an extracellular ligand-binding domain, and when said chimeric DNA is expressed as a membrane bound protein in a selected host cell under conditions suitable for expression, said protein initiates signalling in said host cell.

25. A protein according to Claim 24, wherein said cytoplasmic domain is selected from the group consisting of the CD3 zeta chain, the CD3 eta chain, the CD3 gamma chain, the CD3 delta chain, the CD3 epsilon chain, the gamma chain of a Fc receptor, and a tyrosine kinase.

26. A protein according to Claim 25, wherein the cytoplasmic domain is the gamma chain of the FcεR1 receptor.

27. A protein according to Claim 24 wherein said extracellular binding domain is the heavy chain of an immunoglobulin, by itself or in conjunction with a light chain, or truncated portions of said heavy chain and/or light chain containing ligand binding activity.

28. A protein according to claim 27 wherein said extracellular binding domain is a single-chain antibody, or portion thereof.

5 29. A protein according to Claim 24 wherein said extracellular domain is CD8.

30. A protein according to Claim 24 wherein said extracellular domain is CD4.

31. A mammalian cell comprising as a surface membrane protein, a protein according to Claim 24.

10 32. The mammalian cell of claim 31 wherein said cell is a hematopoietic stem cell.

33. A mammalian cell according to Claim 31, wherein said extracellular domain is bound to a second protein to define a binding site.

15 34. A mammalian cell comprising as a surface membrane protein, a protein according to Claim 27, wherein said cell is a cytotoxic T lymphocyte.

20 35. A mammalian cell comprising as a surface membrane protein, a protein according to Claim 30, wherein said cell is a cytotoxic T lymphocyte.

36. A mammalian cell comprising as a surface membrane protein, a protein according to Claim 27 wherein said cell is substantially free of surface expression of at least one of Class I or Class II MHC.

25 37. A mammalian cell comprising as a surface membrane protein, a protein according to Claim 30, wherein said cell is substantially free of surface expression of at least one of Class I or Class II MHC.

*Rest*  
38. A method for activating cells by means of a secondary messenger pathway, said method comprising:

contacting cells comprising as a surface membrane protein, the protein of claim 24 with a ligand  
5 which binds to said extracellular binding domain and transduces a signal to said cytoplasmic domain,

whereby said secondary messenger pathway is activated.

*Rest*  
39. A method for producing a source of cytotoxic effector cells for killing cells infected with virus or cells expressing tumor antigens comprising introducing the DNA sequence of claim 1 into cells to form modified cells expressing said sequence and transplanting said modified cells into a mammal.  
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40. The method of claim 39 wherein said cells are hematopoietic stem cells.  
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41. The method of claim 39 wherein said extracellular domain is CD4, and said cytoplasmic domain is zeta.

42. The method of claim 38 wherein said extracellular domain is a single-chain antibody, and said cytoplasmic domain is zeta.  
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43. The method of claim 42 wherein said single-chain antibody is specific for HIV env glycoprotein.

44. The method of claim 40 wherein said modified hematopoietic stem cells are transplanted by bone marrow transplantation into said mammal.  
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45. The method of claim 40 wherein said DNA sequence further comprises a genetic marker for determining the

amount of modified hematopoietic stem cells present in the mammal after transplantation.

5 46. A method for treating disease associated with cells infected with virus or tumor cells in a mammal, comprising introducing the DNA of claim 1 into cells to form modified cells expressing said sequence and transplanting said modified cells into a mammal to kill said infected or tumor cells.

10 47. The method of claim 43 wherein said cells are hematopoietic stem cells.

48. The method of claim 44 wherein said modified hematopoietic stem cells are transplanted by bone marrow transplantation into said mammal.

15 49. The method of claim 44 wherein said DNA sequence further comprises a genetic marker and said method further comprises the step of determining the amount of modified hematopoietic cells present in the mammal after transplantation.

20 50. A method for treating disease associated with cells infected with virus or tumor cells in a mammal, comprising introducing the DNA of claim 8 into cells to form modified cells expressing said sequence and transplanting said modified cells into a mammal to kill said cells infected with virus or tumor cells.

25 51. The method of claim 50 wherein said single-chain antibody is reactive with HIV.

52. The method of claim 51 wherein said cytoplasmic domain is zeta.

53. The method of claim 50 wherein said cells are T cells.

54. The method of claim 50 wherein said cells are hematopoietic stem cells.

5 55. The DNA of claim 1 wherein said extracellular binding domain comprises a cell surface receptor joined to a portion of an immunoglobulin.

56. The DNA of claim 55 wherein said cell surface receptor is selected from the group consisting of CD4  
10 and CD8 and said portion of an immunoglobulin is the heavy or light chain of an immunoglobulin.

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B'

Add  
C5